

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Please cancel claims 1-110.

Please add new claims 111-163.

A complete listing of the claims is listed below with proper claim identifiers.

111. (New) A method, of preparing a tissue, comprising:
- providing an uncrosslinked tissue;
  - providing a solution comprising water, a bio-compatible buffer, a cell-impermeant constituent, a cell-permeant constituent, and a radical scavenger;
  - combining the solution with the tissue;
  - lowering the temperature of said solution and said tissue to at least the freezing point of the solution; and
  - irradiating the solution and the tissue.
112. (New) The method of claim 111, wherein the tissue and solution are placed in a package before freezing.
113. (New) The method of claim 111, wherein said solution is degassed.
114. (New) The method of claim 111, wherein irradiating said tissue comprises irradiating the tissue with ionizing radiation.
115. (New) The method of claim 114, further comprising thawing said tissue after the tissue was subjected to extended storage.
116. (New) The method of claim 114, wherein said ionizing radiation is an electron beam.

117. (New) The method of claim 114, wherein said ionizing radiation is gamma radiation.

118. (New) The method of claim 117, wherein said gamma radiation is administered to said tissue at a rate from about 0.38 to 0.45 Mrad/hr.

119. (New) The method of claim 117, wherein a total of from 5,000 to 8,000,000 rads is administered to said tissue.

120. (New) The method of claim 117, further comprising thawing said irradiated tissue, wherein after thawing, the thawed tissue has a denaturation temperature reduced or increased by no more than 3° C from a denaturation temperature of the same type of native tissue.

121. (New) The method of claim 111, further comprising packaging said tissue.

122. (New) The method of claim 121, wherein after said packaging step, said tissue is sterilized.

123. (New) The method of claim 111, further comprising lowering the temperature of said solution and said tissue to at least -18° C.

124. (New) The method of claim 111, wherein the temperature of said solution and said tissue is lowered to at least -130° C.

125. (New) The method of claim 111, wherein the temperature of said solution and said tissue is lowered to at least the glass transition temperature of the solution.

126. (New) The method of claim 111, wherein the temperature of said solution and said tissue is lowered to a cryogenic temperature.

127. (New) The method of claim 111, wherein the tissue is selected from the group consisting of partial organs, heart valve leaflets, aortic roots, aortic walls, pulmonary valves, pulmonary conduits, non-valved conduits, mitral valves, monocusps, tendons, ligaments, fascia, blood vessels, arteries, veins, ureters, diaphragm, pericardium, umbilical cords, dura mater membranes, and tympanic membranes.

128. (New) The method of claim 111, wherein the tissue is selected from the group consisting of heart valves, pulmonary conduits, monocusps, blood vessels, and tendons.

129. (New) The method of claim 111, wherein the tissue is a tendon.

130. (New) The method of claim 111, wherein the tissue is a heart valve.

131. (New) The method of claim 111, wherein the tissue is a pulmonary conduit.

132. (New) The method of claim 111, wherein the tissue is a non-valved conduit.

133. (New) The method of claim 111, wherein the tissue is a monocusp.

134. (New) The method of claim 111, wherein the tissue is decellularized.

135. (New) The method of claim 111, wherein the bio-compatible buffer maintains the pH of said solution from about 6 to about 8 before, during, and after freezing.

136. (New) The method of claim 111, wherein the bio-compatible buffer is isotonic.
137. (New) The method of claim 111, wherein the bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, morpholine propanesulfonic acid buffered saline, tris(hydroxymethyl) aminomethane buffered saline, borate buffered saline, bicarbonate buffered saline, carbonate buffered saline, cacodylate buffered saline, citrate ion buffered saline, and mixtures thereof.
138. (New) The method of claim 111, wherein the bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, tris(hydroxymethyl) aminomethane buffered saline, and mixtures thereof.
139. (New) The method of claim 111, wherein the bio-compatible buffer comprises phosphate-buffered saline.
140. (New) The method of claim 139, wherein the bio-compatible buffer maintains the pH of said solution from about 7 to about 8.
141. (New) The method of claim 139, wherein the bio-compatible buffer maintains the pH of said solution at about 7.4.
142. (New) The method of claim 111, wherein the bio-compatible buffer has a concentration of from 15 to 100 mM in said solution.
143. (New) The method of claim 111, wherein the bio-compatible buffer has a concentration of from 20 to 75 mM in said solution.

144. (New) The method of claim 111, wherein the bio-compatible buffer further comprises sodium chloride.

145. (New) The method of claim 144, wherein the sodium chloride has a concentration of from 0.02 to 0.5 M in said solution.

146. (New) The method of claim 144, wherein the sodium chloride has a concentration of about 0.154 M in said solution.

147. (New) The method of claim 111, wherein the cell-impermeant constituent is selected from the group consisting of proteins, serums, monosaccharides, sucrose, polysaccharides, dextran, agrose, alginate, long-chain polymers, polyvinylpyrrolidone, hydroxyethyl starch, derivatives thereof, and mixtures thereof.

148. (New) The method of claim 111, wherein the cell-impermeant constituent is selected from the group consisting of polyvinylpyrrolidones, hydroxyethyl starches, their derivatives, and mixtures thereof.

149. (New) The method of claim 111, wherein the cell-impermeant constituent comprises a polyvinylpyrrolidone.

150. (New) The method of claim 111, wherein the cell-impermeant constituent comprises from 5 to 30 % of the solution.

151. (New) The method of claim 111, wherein the cell-impermeant constituent comprises from 10 to 14 % of the solution.

152. (New) The method of claim 111, wherein the cell-permeant constituent is selected from the group consisting of alcohols, mannitol, propanediol,

isopropanol, ethanol, t-butanol, glycerol, glycols, ethylene glycol, propylene glycol, trimethylamine acetate, aldoses, ketones, xylose, erythrose, arabinose, ribose, glucose, fructose, galactose, and mixtures thereof.

153. (New) The method of claim 111, wherein the cell-permeant constituent is selected from the group consisting of isopropanol, ethanol, and mixtures thereof.

154. (New) The method of claim 111, wherein the cell-permeant constituent comprises isopropanol.

155. (New) The method of claim 111, wherein the cell-permeant constituent comprises from 5 to 30 % of said solution.

156. (New) The method of claim 111, wherein the cell-permeant constituent comprises about 15 % of said solution.

157. (New) The method of claim 111, wherein the radical scavenger is selected from the group consisting of sodium ascorbate, carotenoids, 1-ascorbic acid, d-isoascorbic acid, sodium sulfite, sodium metabisulfite, sulfur dioxide, nicotinic acid, nicotinic acid amine, cysteine, glutathione, sodium nitrate, sodium nitrite, flavonoids, selenium, alpha-lipoic acids, acetyl cysteine, water-soluble tocopherol derivatives, analogs thereof, isomers thereof, derivatives thereof, and mixtures thereof.

158. (New) The method of claim 111, wherein said radical scavenger is selected from the group consisting of sodium ascorbate, water-soluble derivatives of ascorbate, cysteine, Lazaroids, carotenoids, and mixtures thereof.

159. (New) The method of claim 111, wherein said radical scavenger comprises sodium ascorbate.

160. (New) The method of claim 111, wherein said radical scavenger combines with radicals generated by a gamma irradiation of said solution.

161. (New) The method of claim 111, wherein said radical scavenger has a concentration of from 0.1 to 1.5 M in said solution.

162. (New) The method of claim 111, wherein said radical scavenger has a concentration of about 0.5 M in said solution.

163. (New) The method of claim 111, wherein the solution comprises from 15 to 100 mM of a bio-compatible buffer; from 5 to 30 % of the cell-impermeant constituent comprising polyvinylpyrrolidone; from 5 to 30 % of the cell-permeant constituent comprising isopropanol; and from 0.1 to 1.5 M of a radical scavenger comprising sodium ascorbate.

### **CLAIM STATUS**

Claims 1-110 were cancelled. New claims 111-163 were added. No new matter has been added.

The support for the new claims may be found throughout the specification, including the specified locations noted below with respect to each of the new claims:

Claim 111, see *e.g.*, specification at page 2, paragraphs [0005] and [0006],

Examples 1-5, and original claim 49;

Claim 112, see *e.g.*, original claim 50;

Claim 113, see *e.g.*, original claim 51;

Claim 114, see *e.g.*, original claim 52;

Claim 115, see *e.g.*, original claim 53;

Claim 116, see *e.g.*, original claim 54;

Claim 117, see *e.g.*, original claim 55;

Claim 118, see *e.g.*, original claim 56;

Claim 119, see *e.g.*, original claim 57;

Claim 120, see *e.g.*, original claim 58;

Claim 121, see *e.g.*, original claim 59;

Claim 122, see *e.g.*, original claim 60;

Claim 123, see *e.g.*, original claim 61;

Claim 124, see *e.g.*, original claim 63;

Claim 125, see *e.g.*, original claim 64;

Claim 126, see *e.g.*, original claim 65;

Claims 127-133, see *e.g.*, specification at page 11, paragraph [0033];

Claim 134, see *e.g.*, specification at page 11, paragraph [0035];

Claims 135 and 136, see *e.g.*, specification page 12, paragraph [0037];

Claims 137-141, see *e.g.*, specification page 12, paragraph [0038];

Claims 142 and 143, see *e.g.*, specification page 12, paragraph [0039],

continuing at page 13;

Claims 144-146, see *e.g.*, specification page 13, paragraph [0040];



Claims 147-149, see *e.g.*, specification page 14, paragraph [0043];

Claims 150 and 151, see *e.g.*, specification page 14, paragraph [0044];

Claims 152-154, see *e.g.*, specification page 15, paragraph [0048], continuing at  
page 16;

Claims 155 and 156, see *e.g.*, specification page 16, paragraph [0049];

Claims 157-159, see *e.g.*, specification page 17, paragraph [0053], continuing at  
page 18;

Claims 160, see *e.g.*, specification page 17, paragraph [0052];

Claims 161 and 162, see *e.g.*, specification page 18, paragraph [0054]; and

Claim 163, see *e.g.*, specification page 12 paragraph [0039], continuing at page  
13, page 14, paragraph [0044], page 16, paragraph [0049], and page 18,  
paragraph [0054].